08:40:59 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 07/08/92

Active

Project #: G-33-G16 Cost share #: Rev #: 3
Center # : 10/24-6-Q5169-6A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R01 GM18894-21 Mod #: REV NOA 6/8/92 Document : GRANT
Prime #: Contract entity: GIT

Subprojects ? : N CFDA: 93.821
Main project #: PE #: N/A

Project unit: CHEMISTRY Unit code: 02.010.136
Project director(s):
YU N-T CHEMISTRY (404)894-4007

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 910901 to 930831 (performance) 931130 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	213,133.20
Funded	0.00	213,133.20
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

PROJECT ADMINISTRATION DATA

OCA contact: Kathleen R. Ehlinger 894-4820

Sponsor technical contact

DR. MICHELLE BROIDO
(301)496-7463

NAT INST OF GEN MEDICAL SCIENCES
NATIONAL INSTITUTES OF HEALTH
9000 ROCKVILLE PIKE
BETHESDA, MD 20892

Sponsor issuing office

ANN CALURE, GRANTS MANAGMENT OFFICER
(301)496-7166

NAT INST OF GEN MEDICAL SCIENCES
NATIONAL INSTITUTES OF HEALTH
9000 ROCKVILLE PIKE
BETHESDA, MD 20892

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
ISSUED TO EXTEND TERMINATION DATE FROM 8/31/92 TO 8/31/93.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 11/02/94

Project No. G-33-G16 Center No. 10/24-6-Q5169-6A0

Project Director YU N-T School/Lab CHEMISTRY

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 5 R01 GM18894-21 Contract Entity GIT

Prime Contract No.

Title LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

Effective Completion Date 930831 (Performance) 931130 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	Y	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments
NOTE * USE DHHS FORM FOR PATENT.

Subproject Under Main Project No.

Continues Project No. G-33-G15

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

NOTE: Final Patent Questionnaire sent to PDPI.

G-33-B-2

REVISED

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEA(SERVICE

4260MB

NOTICE OF GRANT AWARD

DATE ISSUED: 06/08/92

GRANT NUMBER: BMT
5 R01 GM18894-21

YPE OF AWARD: RESEARCH
UTHORIZED BY: 42 USC 241 42 CFR 52

TOTAL PROJECT PERIOD:
From 09/01/78 Through 08/31/93

WARDED BY:
NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

tle of Project or Area of Training
LASER-EXCITED RAMAN SPECTROSCOPY OF BIOPOLYMERS

rantee Organization
GEORGIA INST. OF TECHNOLOGY G-33-B-1
225 NORTH AVENUE, N W
ATLANTA, GA 30332

Principal Investigator/Program Director/Awardee
w/ YU, NAI-TENG PHD
GEORGIA INST OF TECH
SCH OF CHEMISTRY AND BIOCHEM
225 NORTH AVE NW
ATLANTA, GA 30332

APPROVED BUDGET		AWARD COMPUTATION	
DR BUDGET PERIOD 09/01/91 Through 08/31/93		DIRECT COSTS ..... $	120,754
ılaries and Wages ..... $ 70,751		INDIRECT COSTS ..... $*	65,969
inge Benefits 7,946			
Total Personnel Costs ..... $	78,697	TOTAL ..... $	186,723
onsultant Costs	4,607		
quipment	15,204	Less Unobligated Balance [Prior Period(s)] $	0
pplies	17,458		
avel - Domestic	1,200	AMOUNT OF THIS AWARD → $	186,723
- Foreign	643		
tient Care - Inpatient		Base Dollars × Rate Percentage = Indirect Costs $*	
- Outpatient			
terations and Renovations			
onsortium/Contractual Costs			
her	2,945		
.....			
ainee Stipends			
ainee Tuition and Fees			
ainee Travel			
TOTAL DIRECT COSTS →	$ 120,754		

hen PHS Prior Approval is required for rebudgeting, submit quests to Grants Management Official below.

SUPPORT RECOMMENDED FOR REMAINDER OF PROJECT PERIOD**

Budget Period	Total Direct Costs (Includes Stipends)	Stipends
22	NONE	

cc: Dr. Yu
J. Dunger
D. Welch
File (3 pgs)

**Subject to availability of funds and satisfactory progress.

MARKS
THIS GRANT IS INCLUDED UNDER EXPANDED AUTHORITIES (PHS GRANTS POLICY STATEMENT REVISED 10/01/90)

PROGRAM ADMINISTRATOR: DR. MICHELLE BROIDO 301-496-7463
GRANTS MANAGEMENT: LUCY CLARKE 301/496-7275

SEE ATTACHED FOR TERMS OF ACCEPTANCE AND ANY ADDITIONAL TERMS AND CONDITIONS.

MS OF ACCEPTANCE: By acceptance of funds awarded under this grant, you grantee acknowledges that it will comply with terms and conditions of the following: (1) applicable regulations cited above; (2) Special provisions noted above under remarks or attached to this notice; (4) 45 CFR Part 74 or 92, as applicable; (5) PHS Grants Administration Manual; (6) PHS Grants [illegible]

—Common Accounting Number	CRS/Entity Identification No.	PHS List No./Object Class Code	Document Number
-8423576	1586002023A1	/41.4E	(08)R1GM18894E

PROGRAM OFFICIAL

. SUE SHAFER, PH.D.
SSOCIATE DIRECTOR FOR
PROGRAM ACTIVITIES
AT. INST. GEN. MED. SCIENCES

PHS Grants Management Official

CAROL L. TIPPERY
GRANTS MANAGEMENT OFFICER
OFFICE ASSOC. DIRECTOR PROGRAM
ACTIVITIES, NIGMS

S 1533
v. 7/88 930608 0830

Copies distributed to Principal Investigator, Program Director or Awardee, and Business Office

GMI 91

5 RO1 GM 18894-21
Dr. Nai-Teng Yu

TERMS AND CONDITIONS

Terms of acceptance: This grant is subject to the terms and conditions incorporated either directly or by reference in the following: (1) The grant program legislation cited on the first page; (2) The grant program regulations cited on the first page; (3) This award notice including terms and conditions, if any, noted below or attached to this notice; (4) PHS Grants Policy Statement including addenda in effect as of the beginning date of the budget period; (5) 45 CFR Part 74 or 92 as applicable. In the event there are conflicting or otherwise inconsistent policies applicable to the grant, the above order of precedence shall prevail. Acceptance of the grant terms and conditions is acknowledged by the grantee when funds are drawn or otherwise obtained from the grant payment system.

1. This award is revised to extend the budget/project period for 12 months in accordance with the grantee's notification letter dated 5/29/92.

2. Any general program income accruing under this grant may be used in accordance with the additional costs alternative described in 45 CFR Part 74 Subpart F [74.42(e)].

3. This award includes $15,204 for the purchase of lasser tubes. These funds are restricted solely for that purpose and may not be rebudgeted.

4. This project is in its final year of recommended support. In the event the project is not renewed or further extended, Public Health Service policy requires submission of the following final reports within 90 days after the grant's final budget period expires:

   (1) Invention Statement (HHS-568)
   (2) Financial Status Report (SF-269)
   (3) Progress Report

   Failure to submit these required reports, when due, may result in the imposition of special award provision or the withholding of support for other eligible projects or activities involving the grantee organization or the individual responsible for the delinquency.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

May 26, 1992

CONTRACTING OFFICER
CRB RM 246
GEORGIA INST. OF TECHNOLOGY
ATLANTA, GA 30332

May 1992 Council
Re: 2R01GM18894-22 4260MB

Dear Mr. Business Official:

The application referenced above was submitted by your institution for review and consideration for funding. The initial review group ranked this application in the lowest third of the applications it reviewed. Consequently, in accordance with our new review procedures, the application was not reviewed by the National Advisory General Medical Sciences Council and cannot receive an award. We will therefore withdraw it administratively. Please be assured that these actions will in no way affect any application that the principal investigator may wish to submit in the future.

If you have any questions, please feel free to contact the program administrator whose name and telephone number are given below.

Sincerely Yours,

W. Sue Shafer, Ph.D.
Associate Director for
Program Activities
National Institute of
General Medical Sciences

The Program Administrator for
your application is:
Michelle S. Broido, Ph.D.
(301) 496-7463

cc: Nai-Teng Yu, Ph.D.

Final Report
of
NIH project: 5 R01 GM18894-21

Title of the Project: Laser-excited Raman Spectroscopy of Biopolymers

Period covered: September 1, 1987 - August 31, 1993.

Institution: Georgia Institute of Technology

The main objectives of the project during the above period are to explore, develop and exploit the information content of resonance Raman spectra of hemoproteins with special emphasis on the detection / interpretation of metal-axial ligand vibrations and their utilization for understanding the mechanisms of protein control of heme reactivity. We have collaborated extensively with Professor Klaus Gersonde of Technical University of Aachen (Germany), and Dr. K. Nagai of Medical Research Council (UK). Prof. Gersonde had provided us with the samples of insect hemoglobin *Chironomus thummi thummi*, while Dr. Nagai supplied us with human mutant Hb's obtained *via* site-directed mutagenesis. These collaborations have been quite fruitful, producing numerous publications as listed below. We have also carried out resonance Raman studies on ligand binding to model compounds, which provide new insight into the exact nature of metal-ligand bonds and their relations with ligand binding affinities. In addition, we have employed near-infrared Fourier Transform Raman technique to study fluorescent or photolabile biomolecules, which were not possible for Raman studies using excitation in the visible region.

The personel who have worked on the project:

Yu, Nai-Teng	P.I.
Gersonde, K.	Research Collaborator (Germany)
Nagai, K.	Research Collaborator (UK)
Lin, S. H.	Graduate Student, completed his Ph.D. degree
Lee, B.-S.	Postdoctoral Research Associate
Lipscomb, L.	Graduate Student, completed her Ph.D. degree
Nie, S.	Postdoctoral Research Associate, becoming Assistant Professor at Indiana University
Tsubaki, M.	Research Collaborator, Summer Res. Associate
Castillo, C.	Graduate Student, completed her Ph.D. degree
Zhang, Fuli	Research Associate
Liu, H.-H.	Graduate Student, completed her M.Sc. degree
Renaud, J.-P.	Vist. Scientist (collaborator) (France)
Tame, Jeremy	Vist. Scientist (collaborator) (UK)
Cai, Ming-Zhi	Research Associate
Chopra, Manu	Graduate Student, will complete his Ph.D. in March 1995
Feng, Sibo	Graduate Student, completed M.Sc. degree; now a Ph.D. candidate at Harvard Univ.
Li, Xiao-Yuan	Research Collaborator (Hong Kong)

Publications resulting from this funded project:

1. Lipscomb, L. A., Lee, B.-S., and Yu, N.-T. **(1993)** "Resonance Raman Investigation of Nitric Oxide Bonding in Iron Porphyrins: Detection of the Fe-NO Stretching Vibration" **Inorg. Chem.** *32*, 281-286.

2. Li, X.-Y., Lipscomb, L. A., Chang, C. K. and Yu, N.-T. **(1992)** "Vibrational Properties of Several Synthetic Heme Models Studied by Raman Related Techniques" in **Proc. SPIE-Laser Applications in Life Sciences,** *1921*, 36-41.

3. Tsubaki, M., Yoshikawa, Ichikawa, Y. and Yu, N.-T. **(1992)** "Effects of Cholesterol Side-Chain Groupd and Adrenodoxin-Binding on the Vibrational Modes of Carbon Monoxide Bound to Cytochrome P-450scc: Implications of the Productive and the Nonproductive Substrate Bindings" **Biochemistry** *31*, 8991-8999.

4. Bormett, R. W., Asher, S. A., Larkin, P. J., Gustafson, W. G., Ragunathan, N., Freedman, T. B., Nafie, L. A., Balasubramanian, S., Boxer, S., Yu, N.-T., Gersonde, K., Noble, R. W., Springer, B. A. and Sligar, S. G. **(1992)** "Selective Examination of Heme Protein Azide Ligand-Distal Globin Interactions by Vibrational Circular Dichroism" **J. Am. Chem. Soc.** *114*, 6864-6867.

5. Nie, S., Lipscomb, L. A. and Yu, Nai-Teng **(1991)** "Surface-Enhanced Hyper-Raman Spectroscopy" **Appl. Spectrosc. Reviews,** *26*(3), 201-274.

6. Hori, H., Tsubaki, M., Yu, N.-T. and Yonetani, T. **(1991)** "Light Absorption, Electron Paramagnetic Resonance and Resonance Raman Characteristics of Nitridochromium(V) Protoporphyrin-IX and Its Reconstituted Hemoproteins" **Biochim. Biophy. Acta,** *1077*, 392-399.

7. Nie, S., and Yu, N.-T. **(1991)** "Surfaced-Enhanced Near-Infrared FT-Raman Scattering of Tetrathiafulvalene Adsorbed on Ag Powder" **J. Raman Spectrosc.** *22*, 489-496.

8. Yu, N.-T. and Nie, S. **(1990)** "Surface-Enhanced Hyper-Raman and Near-IR FT-Raman Studies of Biomolecules" **SPIE**, Vol. 1403, pp. 112-120, **Laser Applications in Life Sciences.**

9. Yu, N.-T., Nie, S., Lipscomb, L. A. **(1990)** "Surface-Enhanced Hyper-Raman Spectroscopy with a Picosecond Laser. New Vibrational Information for Non-Centrosymmetric Carbocyanine Molecules Adsorber on Colloidal Silver" **J. Raman Spectrosc.** *21*, 797-802.

10. Tsubaki, M., Ichikawa, Y., Fujimoto, Y., Yu, N.-T. and Hori, H. **(1990)** "Active Site of Bovine Adrenocortical Cytochrome P-450$_{11b}$ Studied by Resonance Raman and Electron Paramagnetic Resonance Spectroscopies: Distinction from Cytochrome P-450$_{SCC}$" **Biochemistry** 29, 8805-8810.

11. Nie, S., Marzilli, P.A., Marzilli, L. G. and Yu, N.-T. **(1990)** "Near-Infrared Fourier Transform Raman Spectroscopy of Photolabile Organocobalt B12 and Model Compounds. 3. Vibrational Assessment of Factors Affecting the Co-C Bonds in Models." **J. Am. Chem. Soc.** *112*, 6084-91.

12. Lin, S.-H., Yu, N.-T., Tame, J., Shih, D., Renaud, Jean-Paul, Pagnier, J. and Nagai, K. **(1990)** "Effect of the Distal Residues on the Vibrational Modes of the Fe-CO Bond in Hemoglobin Studied by Protein Engineering" **Biochemistry** *29*, 5562-66.

13. Lipscomb, L. A., Nie, S., Feng, S. and Yu, N.-T. **(1990)** "Surface-Enhanced Hyper-Raman Spectroscopy with a Picosecond Laser: Gold and Copper Colloids." **Chem. Phys. Lett.** *170*, 457-461.

14. Lipscomb, L. A., Nie, S., Feng, S. and Yu, N.-T. **(1990)** "Surface-Enhanced Hyper-Raman Spectroscopy with a Picosecond Laser: Gold and Copper Colloids." **Chem. Phys. Lett.** *170*, 457-461.

15. Liu, H.-H., Lin, S.-H. and Yu, N.-T. **(1990)** "Resonance Raman Enhancement of Phenyl Ring Vibrational Modes in Phenyl Iron Complex of Myoglobin" **Biophys. J.** *57*, 851-856.

16. Nie, S, Lipscomb, Feng, S. and Yu, N.-T. **(1990)** "Resonant and Nonresonant Surface-Enhanced Hyper-Raman Spectroscopy with a Picosecond Laser. Effect of the Excitation Pulse Width" **Chem. Phys. Lett.** *167*, 35-40.

17. Nie, S., Marzilli, L.G., and Yu, N.T. **(1989)** "Near-Infrared Fourier Transform Raman Spectroscopy of Photolabile Organocobalt B_{12} and Model Compounds. 1. Detection of the Cobalt-Carbon Stretching Mode in the Solid State and in Solution" **J.Am. Chem. Soc.** *111*, 9256-58.

18. Yu, N.-T., Lin, S.-H., Chang, C.K. and Gersonde, K. **(1989)** "Resonance Raman Enhancement of the Mn-N-O Bending Mode in Nitrosyl Manganese"Strapped" and "Open" Heme Complexes" **Biophys. J.** *55*, 1137-1144.

19. Gersonde, K., Yu, N.-T., Lin, S.-H., Smith, K. M. and Parish, D. W. **(1989)** "Resonance Raman Assignment and Evidence for Noncoupling of Individual 2- and 4-Vinyl Vibrational Modes in a Monomeric Cyanomethemoglobin" **Biochemistry,** *28*, 3960-3966.

20. Lin, S.-H., Yu, N.-T. and Gersonde, K. **(1988)** "Resonance Raman Evidence for an Unusually Strong Exogenous Ligand-Metal Bond in a Monomeric Nitrosyl Manganese Hemoglobin" **FEBS Lett.** *229*, 367-371.

21. Tanaka, T., Yu, N.-T. and Chang, C.K. **(1987)** "Resonance Raman Studies of Sterically Hindered Cyanomet "Strapped" Hemes: Effects of Ligand Distortion and Base Tension on Iron-Carbon Bond" **Biophys. J.** *52*, 801-805.

22. Tsubaki, M., Hori, H., Hotta, T., Hiwatashi, A., Ichikawa, Y. and Yu, N.-T. **(1987)** "Influence of Heme-Surrounding Amino Acid Residues on Manganese (V)-Nitrido Bond in Manganese-Substituted Hemoproteins: Resonance Raman Evidence for Porphyrin Core Expansion and Reduction of Manganese (V)-Nitrido Stretching Force Constant" **Biochemistry** *26*, 4980-86.

23. Nagai, K., Luisi, B., Shih, D., Miyazaki, G., Imai, K., Poyart, C., DeYoung, A., Kwiatkowsky, L., Noble, R. W., Lin, S.-H. and Yu, N.-T. **(1987)** "Distal Residues in the Oxygen Binding Site of Hemoglobin Studied by Protein Engineering" **Nature**, *329*, 858-860.

24. Yu, N.-T. and Kerr, E. A. **(1987)** "Vibrational Modes of Coordinated CO, CN-, NO and O_2" *in* Biological Applications of Raman Spectroscopy (Spiro, T. G., Ed.) John Wiley & Sons.

25. Gersonde, K., Yu, N.-T., Kerr, E. A., Smith, K. M. and Parish, D. W. **(1987)** "Heme-Rotational Disorder in Monomeric Allosteric Cyano-Met Inset Hemoglobins Monitored by Resonance Raman Spectroscopy" **J. Mol. Biol.** *194*, 545-556.

26. Gersonde, K., Yu, N.-T., Kerr, E. A., Smith, K. M. and Parish, D. W. **(1987)** "Heme-Rotational Disorder in Monomeric Allosteric Cyano-Met Inset Hemoglobins Monitored by Resonance Raman Spectroscopy" **J. Mol. Biol.** *194*, 545-556.

27. Thompson, H. M., Yu, N.-T. and Gersonde, K. **(1987)** "Resonance Raman Evidence for the Mechanism of the Allosteric Control of O_2-Binding in a Cobalt-Substituted Monomeric Insect Hemoglobin" **Biophys. J.** *51*, 289-295.

Significant findings may be summarized as follows:

(a) We made the first comparison of the $\nu(Fe^{II}\text{-}NO)$ stretching frequencies in iron porphyrins (~527 cm^{-1}) and hemoproteins (~554 cm^{-1}) to assess the protein's effect on the Fe-NO bond. The electronic trans effect was investigated by preparing complexes with N-methylimidazole, pyridine, and tetrahydrofuran as proximal ligands, and steric effects were assessed by comparing hindered (1,2-dimethylimidazole) and unhindered (N-methylimidazole) axial bases. Surprisingly, the Fe^{II}-NO bond strength proved insensitive to alterations in the trans base. The results were contrasted with Fe^{II}-CO, Fe^{II}-O_2, and Fe^{II}-CN studies, in which the iron-ligand bond strength was found to be dramatically affected by properties of the proximal ligand.

(b) The nitridochromium (V) porphyrin complex was produced by UV irradiation of the azide complexes of the chromium (III)-protoporphyrin-IX. The Cr(V)-N stretching mode was observed at 1010 $cm^{-1}$by resonance Raman spectroscopy. The force constant of 6.7 mdyn/A for the Cr(V)-N bond was found, consistent with that for the metal-nitrogen triple bond.

(c) Surface-Enhanced hyper-Raman Spectroscopy: We have demonstrated the effect of excitation pulse width on hyper-Raman intensity. We have shown that SEHR technique provides new vibrational information for non-centrosymmetric molecules. Surface-enhanced hyper-Raman signals from gold and copper colloids were demonstrated.

(d) Raman Evidence for Non-coupling of individual 2- and 4- Vinyl Vibrational Modes in Cyano-met Hb: By studying cyano-met CTT Hbs reconstituted with protoheme-IX selectively deuterated at vinyl and 2,4-vinyls, pemptoheme, isopemtoheme, and symmetric hemes (protoheme-III and -XIII),we have obtained unequivocal evidence that the highly localized vinyl C=C stretching vibrations at the 2 and 4 positions are non-coupling and inequivalent.

(e) The Origin of the Distal Steric Effect in Carbonmonoxy Hemoglobin: We have obtained new insight into the origin of the distal effect in Hb·CO. Dr. Nagai provided us with human mutant Hbs having: (1) His(αE7) replaced by Gln or Gly; (2) His(βE7) by Gln, Val, Gly or Phe; (3) Val(αE11) by Ala, Leu or Ile; (4) Val(βE11) by Ala, Met, Leu or Ile; and (5) Phe(CD1) by Gly or Tyr. The effects of

these mutations on the vibrational properties of the Fe-C and C-O bonds in carbonmonoxy Hb A have been studied by Soret-excited resonance Raman spectroscopy. It is concluded that the origin of the distal steric effect that causes the off-axis CO bonding is not the steric bulk of the E-7 residue. Instead, it may be the repulsive polar interactions between the lone-pair electrons of the N_ϵ and those of the carbonyl oxygen.

(f) Resonance Raman Probe of Heme-Rotational Dosorder in Cyano-met CTT Hbs: We have identified two Fe-C-N Bending Vibrations attributable to heme-rotational components in deutero-IX and meso-IX reconstituted CTTs.

(g) Near-Infrared FT-Raman Spectroscopy for the Studies of Photolabile and Fluorescent Biomolecules: For the first time, we reported the detection of the stretching vibration of the Co-C bond in photolabile B_{12} and model compounds by near-IR excited FT-Raman spectroscopy. This opens up the possibility of directly monitoring the Co-C bond in various B_{12} complexes.

(h) Assessment of Factors Affecting the Co-C Bonds in B_{12} Models: We have assess the importance of various factors such as trans electronic effect, trans steric effect, environmental effect, which influence the Co-C bond stretch (hence Co-C bond strength).

(i) No Change in Nitrosyl Bonding Geometry in Mb·NO between 20 ^{0}C and 77 ^{0}K: By single crystal EPR spectroscopy, the Fe-N-O bond angle in Mb.NO was found to change by temperature from 153^0 (at 20 ^{0}C) to 109^0 (at 77 ^{0}K). Unexpectedly, we found no change in the Fe-NO stretch at 554 cm^{-1} between the two temperatures, indicating no change in nitrosyl bonding geometry, The discrepancy simply indicates that EPR data need to be re-interpreted.

(j) Unusual Ligand Binding to Liver Fluke Dd Hemoglobin: We have detected the Fe(II)-NO, Fe(II)-CO, and Fe(II)-O_2 stretching vibrations of Dd Hb at 567, 571 and 478 cm^{-1}, respectively. Compared with the Fe(II)-NO stretching mode of Mb.NO and Hb.NO at 554 and 551 cm^{-1}, the $\nu(Fe^{II}\text{-NO})$ frequency for Dd Hb.NO is ~15 cm^{-1} higher. On the other hand, the Fe^{II}-CO stretch of Dd Hb.CO is ~32 cm^{-1} lower than that of Mb·CO (507 cm^{-1}) and HbA·CO (512 cm^{-1}). The replacement of distal His by Tyr in Dd Hb indeed has a profound effect on the $\nu(Fe^{II}\text{-NO})$ and $\nu(Fe^{II}\text{-CO})$ vibrations.

Reprinted from Inorganic Chemistry, 1993, 32.

Resonance Raman Investigation of Nitric Oxide Bonding in Iron Porphyrins: Detection of the Fe–NO Stretching Vibration

Leigh Ann Lipscomb,† Bao-Shiang Lee,† and Nai-Teng Yu*,†,‡

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332, and Department of Chemistry, The Hong Kong University of Science and Technology, Kowloon, HK

Received September 20, 1991

With Soret-excited resonance Raman spectroscopy (RRS), we have detected the $\nu(Fe^{II}–NO)$ and $\nu(Fe^{III}–NO)$ stretching vibrations for several iron porphyrins, including octaethylporphyrin (OEP), tetraphenylporphyrin (TPP), heme-5, SP-13, SP-14, and SP-15. This result enables us to make the first comparison of the $\nu(Fe^{II}–NO)$ stretching frequencies in iron porphyrins ($\sim$527 cm^{-1}) and hemoproteins ($\sim$554 cm^{-1}), which is of interest if the protein's effect on the Fe–NO bond is to be assessed. Solvent effects on the $Fe^{II}–NO$ bond strength were significant; for Fe^{II}(OEP)(pyridine)(NO), the $\nu(Fe^{II}–NO)$ stretching frequency was observed 5 cm^{-1} higher in CH_2Cl_2 (527 cm^{-1}) than in benzene (522 cm^{-1}). The electronic trans effect was investigated by preparing complexes with *N*-methylimidazole, pyridine, and tetrahydrofuran as proximal ligands, and steric effects were assessed by comparing hindered (1,2-dimethylimidazole) and unhindered (*N*-methylimidazole) axial bases. Interestingly, the $Fe^{II}–NO$ bond strength proved insensitive to alterations in the trans base. These results are contrasted with previous $Fe^{II}–CO$, $Fe^{II}–O_2$, and $Fe^{III}–CN$ studies, in which the iron–ligand bond strength was found to be dramatically affected by properties of the proximal ligand.

Introduction

Nitrosyl complexes have been extensively investigated by X-ray diffraction,[1-4] infrared,[4,5] electron spin resonance (EPR),[6-10] visible absorption,[11-13] extended X-ray absorption fine structure (EXAFS),[14] and resonance Raman spectroscopy (RRS).[15-23] It is known that nitric oxide (NO) binds to both ferric and ferrous hemes with an unusually high affinity.[24] With RRS, the Fe–NO bond can be readily monitored in terms of its stretching mode. The first detection of $\nu(Fe^{II}–NO)$ in hemoproteins (554 cm^{-1}, ferrous HbA–NO) was reported in 1977 by Chottard and Mansuy.[15] Somewhat later, Tsubaki and Yu[22] used RRS to study $\nu(Fe^{II}–NO)$ (551 cm^{-1}) and $\nu(N–O)$ ($\sim$1623 cm^{-1}) in nitrosylhemoglobin A (HbA) and myoglobin. For ferric hemoproteins, Benko and Yu[25] were the first to observe $\nu(Fe^{III}–NO)$ and δ-$(Fe^{III}–N–O)$ in nitrosyl complexes of HbA and horseradish peroxidase. Recently, Choi et al.[26] have reported the resonance Raman detection of $\nu(Fe^{II}–NO)$ for the five-coordinate Fe^{II}(TPP)(NO). However, no infrared or resonance Raman detection of $\nu(Fe^{II}–NO)$ and $\nu(Fe^{III}–NO)$ has been reported for the six-coordinate Fe^{II}(porphyrin)(base)(NO) in the literature. Such information is essential for assessing the protein's influence on the Fe–NO moiety, since the protein systems are also six-coordinate (with histidine as the proximal base).

In this paper, we describe Soret-excited RRS studies of nitric oxide complexes of ferric and ferrous porphyrins. With nitric oxide isotope substitution, the $\nu(Fe^{III}–NO)$ and $\nu(Fe^{II}–NO)$ stretching modes have been clearly identified at $\sim$602 and $\sim$524 cm^{-1}, respectively. The protein's influence on the $\nu(Fe^{II}–NO)$ stretching frequency ($\sim$30 cm^{-1}) is indeed significant. We have also investigated the effects of the solvent and trans base on the $Fe^{II}–NO$ bond. Trans bases of different strengths (*N*-methylimidazole, pyridine, tetrahydrofuran) were studied, and sterically hindered (1,2-dimethylimidazole) and unhindered (*N*-methylimidazole) bases were also compared. We were surprised to find that the $Fe^{II}–NO$ bond is insensitive to the nature of the trans ligand. Solvent effects on the $\nu(Fe^{II}–NO)$ stretching frequency were investigated by comparing complexes of Fe^{II}(OEP)(pyridine)(NO) in benzene, tetrahydrofuran, carbon tetrachloride, chloroform, and methylene chloride. The $\nu(Fe^{II}–NO)$ stretching frequency was generally observed to increase for solvents of higher dipole moment.

Experimental Section

Iron(III) octaethylporphyrin (OEP) chloride was obtained from Strem Chemicals (Newburyport, MA), while iron(III) *meso*-tetraphenylporphyrin (TPP) bromide was purchased from Midcentury (Posen, IL). Both porphyrins were used without further purification. Heme 5, as well

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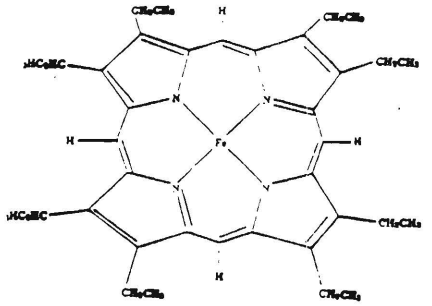
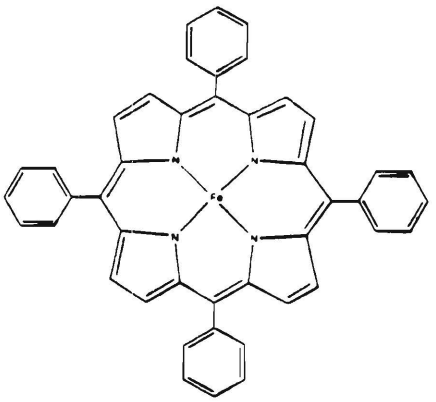
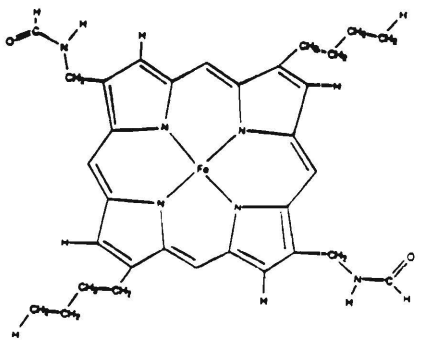


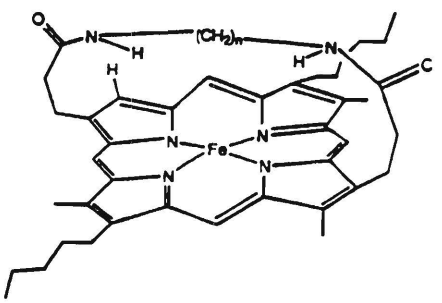
Iron Octaethylporphyrin



Iron Tetraphenylporphyrin



Heme 5



FeSP-13 n=5
FeSP-14 n=6
FeSP-15 n=7

Figure 1. Chemical structures of iron porphyrins.

as the strapped hemes (FeSP-13, FeSP-14, FeSP-15), was synthesized according to literature methods.[27] The NO complexes of these ferric porphyrins (200 μM) were prepared with tetrahydrofuran (THF, 0.10 M), *N*-methylimidazole (*N*-MeIm, 0.15 M), 1,2-dimethylimidazole (1,2-DiMeIm, 0.15 M), and pyridine (0.15 M) as proximal bases in CH_2Cl_2 solution. For preparation of the ferrous complex, methanol (0.1 M) was added to the porphyrin/CH_2Cl_2/base solution prior to the addition of the NO gas; methanol is known to act as a catalyst in the autoreduction of ferric porphyrins by NO.[20] The sample solution was placed in a quartz Raman cell, which was immediately sealed and evacuated. Nitric oxide (from Aldrich, Milwaukee, WI) was then introduced at a pressure of ~1 atm. The isotope-substituted complexes were prepared similarly with $^{15}N^{16}O$ (Bio-Rad Laboratories, Richmond, CA), and $^{15}N^{18}O$ (ICON Services, Summit, NJ).

All reagents were purified prior to use. Methylene chloride was stirred over concentrated sulfuric acid, neutralized with a 10% $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and distilled from phosphorus pentoxide. Pyridine and *N*-MeIm were distilled from KOH, while 1,2-DiMeIm and methanol were distilled from Na and Mg, respectively. Reagent grade tetrahydrofuran was used as received from Fisher Scientific (Fair Lawn, NJ).

The multichannel laser Raman system has been described previously.[28] The 406.7-nm line of a Spectra-Physics (Mountain View, CA) Model 171 krypton ion laser was employed for excitation. The laser used to obtain each spectrum is listed in the figure captions. To minimize photodissociation and localized heating, the Raman cell was kept spinning throughout the entire data acquisition process. The entrance slit of the monochromator had a width and height of 100 μm and 1 cm, respectively. Fenchone was used to calibrate the spectra; peak positions are considered accurate to ±2 cm^{-1}.

Results and Discussion

Identification of the ν(Fe–NO) Stretching Vibration. We have studied the effects of nitric oxide isotope substitution on the low-(100–700 cm^{-1}) and high (1300–1900 cm^{-1}) frequency regions of Fe^{II}(OEP)(THF)(NO) spectra. (See Figure 1 for structures of all the iron porphyrins studied in this work.) The substitutions reveal one isotope-sensitive line at 527 cm^{-1} for Fe^{II}(OEP)(THF)-($^{14}N^{16}O$), which shifts to 519 cm^{-1} ($^{15}N^{16}O$) and to 512 cm^{-1} ($^{15}N^{18}O$) (see Figure 2). It is clear that this line corresponds to the 554-cm^{-1} mode in nitrosyl HbA assigned by others[15] as ν-(Fe^{II}–NO). Careful examination of the spectral region from 400 to 500 cm^{-1} reveals no $\delta(Fe^{II}–N–O)$ bending mode for any of the porphyrins studied.

Because the autoreduction of Fe^{III}(OEP)(pyridine)(NO) to Fe^{II}(OEP)(pyridine)(NO) is very slow (~48 h), we were able to study the effects of nitric oxide isotope substitution on the low- and high-frequency regions of Fe^{III}(OEP)(pyridine)(NO) spectra. In Figure 3, an isotope-sensitive line appears at 602 cm^{-1} for Fe^{III}(OEP)(pyridine)($^{14}N^{16}O$), which shifts to 596 cm^{-1} for Fe^{III}-(OEP)(pyridine)($^{15}N^{16}O$). Other ferric porphyrins studied include Fe^{III}(heme-5)(pyridine)(NO) (602 cm^{-1}), Fe^{III}(SP-14)-(pyridine)(NO) (603 cm^{-1}), and Fe^{III}(SP-15)(pyridine)(NO) (601 cm^{-1}). As was the case for the ferrous porphyrins, no bending modes were observed in the spectra. This result is not surprising in view of the linearity of the Fe^{III}–NO system, which is expected on the basis of the total number of electrons (6) in the metal d and ligand π^* orbitals.[29] It is of interest to point out that previous RRS studies of sterically hindered carbonmonoxy strapped hemes[30] indicated that distortion of the Fe^{II}–C–O linkage enhances the intensity of $\delta(Fe^{II}–C–O)$ relative to that of $\nu(Fe^{II}–CO)$. While the Fe^{III}–NO and Fe^{II}–CO systems are isoelectronic, the Fe^{II}–CO bond length of 1.77 Å observed for Fe^{II}(TPP)(pyridine)-

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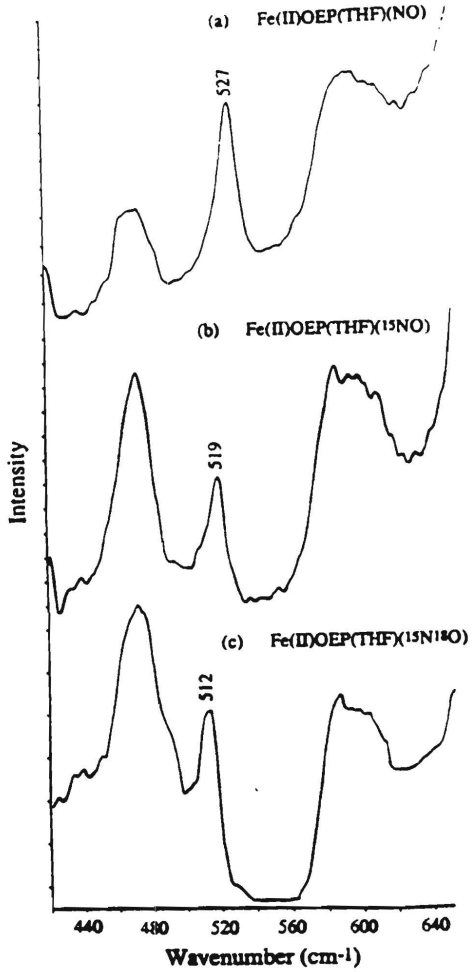

Figure 2. Nitric oxide isotope effects on the low-frequency region (420–650 cm^{-1}) spectra of Fe^{II}(OEP)(THF)(NO). λ_{ex} = 406.7 nm; laser power = 40 mW at the sample. Because only small portions of the spectra are displayed in each figure, it should be mentioned that all features of our RRS data are in good agreement with those previously published for metallooctaethylporphyrins.[60]

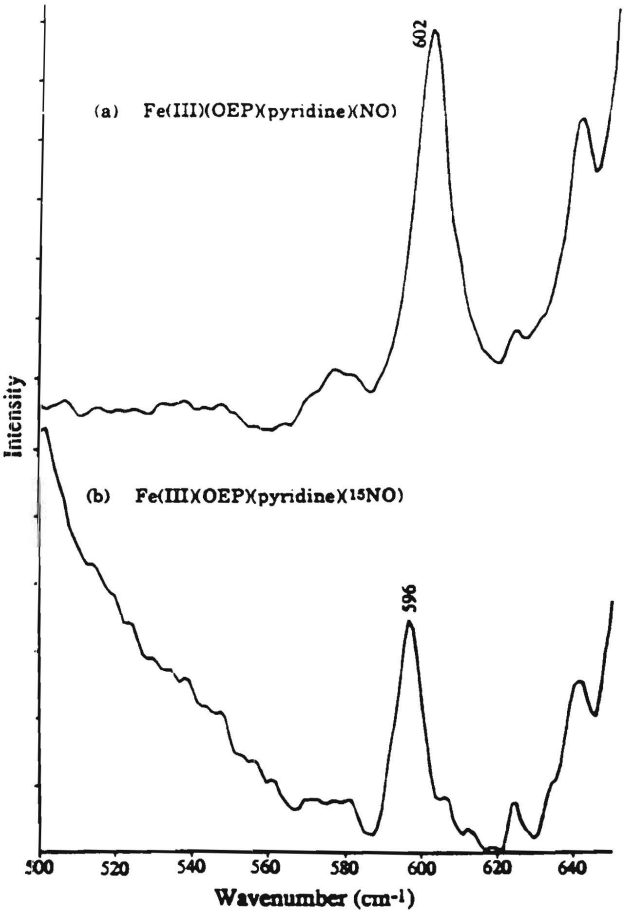

Figure 3. Nitric oxide isotope effects on the low-frequency region (500–650 cm^{-1}) spectra of Fe^{III}(OEP)(pyridine)(NO). λ_{ex} = 406.7 nm; laser power = 40 mW at the sample.

(CO)[31] is much longer than the Fe^{III}–NO bond investigated in this paper, because the observed ν(Fe^{II}–CO) stretch (486 cm^{-1})[30] is much lower than ν(Fe^{III}–NO) of 602 cm^{-1} reported here. It is well-known[29] that the stretching frequency can be correlated with the force constant and bond length. We suggest that the Fe^{III}–NO bond of Fe^{III}(SP-14)(pyridine)(NO) and Fe^{III}(SP-15)-(pyridine)(NO) is short enough to prevent geometric distortion of the Fe^{III}–NO linkage by the strap. Therefore, Fe^{III}–NO maintains its preferred linear configuration, and δ(Fe^{III}–N–O) is not enhanced.

We have also identified ν(Fe^{II}–NO) and ν(Fe^{III}–NO) for several other iron porphyrins, and all of the results are given in Table I. It should be mentioned that unsuccessful attempts to observe ν(Fe^{II}–NO) in porphyrins were previously reported by Stong et al.[20] Both the excitation wavelength (454.5 nm) and detector (photomultiplier tube) were different from those employed in this study.

Comparison of [Fe^{III}–NO] with the Isoelectronic [Fe^{II}–CO] System. In the absence of distal steric hindrance, both the Fe^{III}–NO and Fe^{II}–CO moieties are known to adopt linear configurations normal to the heme plane. X-ray and neutron scattering crystal structures of carbonmonoxy adducts of hemoproteins[33-42] reveal that nonbonded contacts in the heme pocket force the bound CO out of its preferred linear configuration. Li and Spiro[43] have suggested that these protein-induced distortions result mainly in tilting rather than bending of the Fe^{II}–CO unit. Yu et al.,[30] in studies of sterically hindered carbonmonoxy strapped hemes, observed that distortions in the Fe^{II}–CO linkage resulted in an increase in the ν(Fe^{II}–CO) stretching frequency. It is of interest to ask whether or not similar distortions occur in NO adducts of ferric hemoproteins.

Table I. Position of ν(Fe–NO) for Ferric and Ferrous Porphyrins

ferrous porphyrins	ν(Fe^{II}–NO) (cm^{-1})
Fe^{II}(OEP)(pyridine)(NO)	527
Fe^{II}(TPP)(pyridine)(NO)	527
Fe^{II}(heme-5)(pyridine)(NO)	528
Fe^{II}(SP-13)(pyridine)(NO)	528
Fe^{II}(SP-14)(pyridine)(NO)	526
Fe^{II}(SP-15)(pyridine)(NO)	524
ferric porphyrins	**ν(Fe^{III}–NO) (cm^{-1})**
Fe^{III}(OEP)(pyridine)(NO)	602
Fe^{III}(heme-5)(pyridine)(NO)	602
Fe^{III}(SP-14)(pyridine)(NO)	603
Fe^{III}(SP-15)(pyridine)(NO)	601

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The similar $\nu(Fe^{III}–NO)$'s for ferric HRP–NO (604 cm^{-1}), ferric Mb–NO (595 cm^{-1}),[25] and Fe^{III}(OEP)(pyridine)(NO) (602 cm^{-1}) indicate that the protein's effect on the Fe^{III}–NO bond is small, compared with the Fe^{II}–CO system. Previously, the $\nu(Fe^{II}–CO)$ stretching frequency was observed 16 cm^{-1} higher for MbCO (512 cm^{-1})[44] than for Fe^{II}(OEP)(*N*-methylimidazole)(CO) (496 cm^{-1}).[32] For Mb, the relative stretching frequencies of $\nu(Fe^{III}–NO)$ (595 cm^{-1})[25] and $\nu(Fe^{II}–CO)$ (512 cm^{-1})[44] suggest that the Fe^{III}–NO bond is stronger than the Fe^{II}–CO bond. However, to avoid being misled by mass effects, we calculated the $\nu(Fe^{II}–CO)$ stretching frequency (for Mb–CO) on the basis of corrected mass normalized to the Fe^{III}–NO system. We then obtained a value of 501 cm^{-1} for $\nu(Fe^{II}–CO)$. These frequency numbers (501 cm^{-1} for Fe^{II}–CO, 595 cm^{-1} for Fe^{III}–NO) do indeed correlate with a force constant that is highest for the Fe^{III}–NO bond. While X-ray data for nitrosyl adducts of ferric hemoproteins are unavailable, a comparison of the stretching frequencies indicates that the Fe^{III}–NO bond is strongest and hence the shortest. Evidently the strength of the Fe^{III}–NO bond renders it less susceptible to protein distortions. It is plausible that this bond is short enough not to sterically interfere with bulky amino acid residues near the binding site.

Protein Effect. The results presented here facilitate the first comparison of the $\nu(Fe^{II}–NO)$ stretching frequencies in model compounds and hemoproteins. For Fe^{II}(OEP)(*N*-MeIm)(NO), $\nu(Fe^{II}–NO)$ was detected at 524 cm^{-1}, ca. 30 cm^{-1} lower than that observed for nitrosyl HbA (~554 cm^{-1}).[15] An interesting observation evolves from the comparison of protein effects on Fe^{II}–NO and Fe^{II}–O_2. While both of these moieties maintain bent geometries, the protein effect on Fe^{II}–O_2[23,49] is much smaller than that on Fe^{II}–NO, and the NO binding to Fe^{II}–heme in Hb is ~1000 times stronger than O_2 binding.[24]

To obtain a more definitive assessment of the protein's effect on the Fe^{II}–NO bond, we have carried out resonance Raman studies of Fe^{II}(PP)(*N*-MeIm)(NO) (PP = protoporphyrin IX) in aqueous solution at alkaline pH. Because iron protoporphyrin IX is present in Hb and Mb, comparisons of the $\nu(Fe^{II}–NO)$ stretching frequency in Mb–NO and Fe^{II}(PP)(*N*-MeIm)(NO) should reflect only the effects of the protein. Unfortunately, slight background fluorescence prevented observation of $\nu(Fe^{II}–NO)$ for the protoporphyrin IX system. But the comparisons we have made between Fe^{II}(OEP)(*N*-MeIm)(NO) and Mb–NO and Hb–NO are still useful, because alterations in the heme group have only a slight effect on $\nu(Fe^{II}–NO)$ (see Table I). Furthermore, the $\nu(Fe^{II}–CO)$ stretching frequencies reported for Fe^{II}(PP)(*N*-MeIm)(CO) (497 cm^{-1})[45] and Fe^{II}(OEP)(*N*-MeIm)(CO) (496 cm^{-1})[32] are very similar.

Trans Effect. Assuming that the Fe^{II}–N–O geometry is not affected by a change in the trans ligand, the $\nu(Fe^{II}–NO)$ stretching frequency may be correlated with the Fe^{II}–NO bond strength (or bond length). In this work, we have examined the influence of trans ligands on the $\nu(Fe^{II}–NO)$ stretching frequency. The ligands studied were *N*-methylimidazole (N-MeIm), pyridine, and tetrahydrofuran (THF). It is also interesting to compare the $\nu(Fe^{II}–NO)$ stretching frequency in complexes with *N*-methylimidazole and 1,2-dimethylimidazole (1,2-DiMeIm) as proximal bases. The methyl group in the 2-position of 1,2-DiMeIm sterically interferes with the porphyrin plane, thereby lengthening the Fe–N_t bond (N_t represents the N atom of the proximal base). Iron porphyrin complexes with 1,2-DiMeIm provide an excellent model for the tense (or low affinity) state of hemoglobin.[46] Results for Fe^{II}(OEP)(*N*-MeIm)(NO), Fe^{II}(OEP)(1,2-DiMeIm)(NO), and Fe^{II}(OEP)(pyridine)(NO) are shown in Figure 4, while the spectrum of Fe^{II}(OEP)(THF)(NO) is displayed in Figure 1. Interestingly, $\nu(Fe^{II}–NO)$ proved insensitive to both electronic and steric trans effects. Kincaid et al.[47] have previously noted similar behavior of $\nu(Fe^{II}–NO)$ of nitrosyl adducts of ferrous cytochrome P450cam. The authors report that the $\nu(Fe^{II}–NO)$ stretching frequency was not altered by proximal cysteine ligation. Apparently, bending of the Fe–N–O linkage substantially increases the energy of the d_{xy}-π orbitals,[47,48] thus rendering the transfer of a lone pair on the axial sulfur energetically unfavorable.

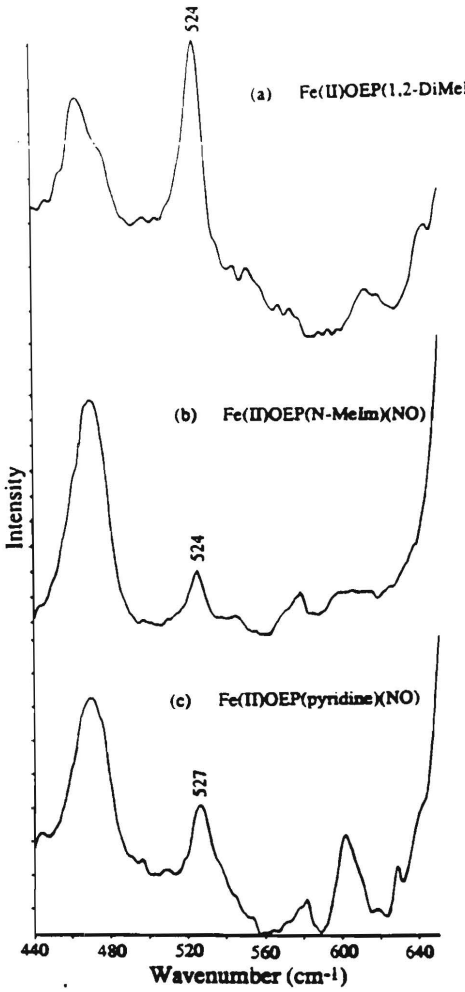

Figure 4. Effects of steric and electronic properties of the trans ligand on $\nu(Fe^{II}–NO)$ in the low-frequency region (440–650 cm^{-1}) spectra of nitrosyliron(II) OEP complexes. The laser power was 40 mW (at the sample) in (a) and (c) and 30 mW in (b). λ = 406.7 nm.

To make certain that we were not studying the bis(nitrosyl) adduct, Fe^{II}(OEP)$(NO)_2$, we prepared this adduct and studied its Raman and absorption spectra. In the absence of any base, Fe^{II}(OEP)$(NO)_2$ can be readily formed under our experimental conditions.[49] The absorption spectrum obtained from this complex was significantly different from that obtained for the Fe^{II}(OEP)(base)(NO) complexes (data not shown). Additionally, we observed an isotope-sensitive line at 519 cm^{-1} in the resonance Raman spectrum of Fe^{II}(OEP)$(^{14}N^{16}O)_2$; this line shifts to 514 cm^{-1} upon substitution by $^{15}N^{16}O$. The $\nu(Fe^{II}–NO)$ stretching frequency detected for Fe^{II}(OEP)$(NO)_2$ (519 cm^{-1}) is indeed significantly different from that observed for Fe^{II}(OEP)(base)(NO) (~527 cm^{-1}).

Additionally, we have monitored the $\nu(Fe^{II}–NO)$ stretching frequency under conditions which we can be positive that the trans ligand is coordinated. Fe^{II}(OEP)(pyridine)(NO) was prepared in neat pyridine, and $\nu(Fe^{II}–NO)$ was detected at 526 cm^{-1} (spectrum not shown). $\nu(Fe^{II}–NO)$ was also observed at

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Table II. Comparison of the Trans Effects for CO, O_2, CN, and NO Complexes of Iron Porphyrins: Results from Resonance Raman Spectroscopy

complex	$\nu(Fe^{II}–CO)$ (cm^{-1})	ref
Fe^{II}(TpivPP)(THF)(CO)	527	32
Fe^{II}(TpivPP)(pyridine)(CO)	486	32
Fe^{II}(TpivPP)(*N*-MeIm)(CO)	489	32
Fe^{II}(TpivPP)(1,2-DiMeIm)(CO)	496	32
complex	**$\nu(Fe^{II}–O_2)$ (cm^{-1})**	**ref**
Fe^{II}(TpivPP)(*N*-MeIm)(O_2)	571	51
Fe^{II}(TpivPP)(1,2-DiMeIm)(O_2)	561	51
complex	**$\nu(Fe^{III}–CN)$ (cm^{-1})**	**ref**
Fe^{III}(SP-15)(*N*-MeIM)(CN)	447	52
Fe^{III}(SP-15)(pyridine)(CN)	456	52
Fe^{III}(SP-15)(1,2-DiMeIm)(CN)	446	52
complex	**$\nu(Fe^{II}–NO)$ (cm^{-1})**	**ref**
Fe^{II}(OEP)(THF)(NO)	527	this work
Fe^{II}(OEP)(*N*-DiMeIm)(NO)	524	this work
Fe^{II}(OEP)(1,2-DiMeIm)(NO)	524	this work
Fe^{II}(OEP)(pyridine)(NO)	527	this work

525 cm^{-1} for both Fe^{II}(OEP)(*N*-methylimidazole)(NO) (in neat *N*-methylimidazole) and Fe^{II}(OEP)(1,2-dimethylimidazole)-(NO) (in neat 1,2-dimethylimidazole). Clearly, these new results unambiguously demonstrate that $\nu(Fe^{II}–NO)$ is insensitive to electronic/steric properties of the trans base.

This lack of an electronic trans effect has been noted previously for the Mn^{II}–NO system. Yu et al.[50] reported almost no difference in the $\nu(Mn^{II}–NO)$ stretching frequencies observed for Mn^{II}-(heme-5)(N-MeIm)(NO) (629 cm^{-1}) and Mn^{II}(heme-5)(piperidine)(NO) (630 cm^{-1}). In contrast, other ligand systems such as Fe^{II}–CO[32], Fe^{II}–O_2,[51] and Fe^{III}–CN,[52] are known to exhibit significant trans effects. In Table II, we compare the sensitivities of the Fe^{II}–CO, Fe^{II}–O_2, Fe^{III}–CN, and Fe^{II}–NO bonds to alterations in the proximal base. Results in Table II indicate that, while the $\nu(Fe^{II}–CO)$ stretching frequency for Fe^{II}(TpivPP)-(pyridine)(CO) is 41 cm^{-1} lower than that of Fe^{II}(TpivPP)(THF)-(CO), the position of $\nu(Fe^{II}–NO)$ is identical (527 cm^{-1}) for Fe^{II}(OEP)(pyridine)(NO) and Fe^{II}(OEP)(THF)(NO). These resonance Raman results are supported by X-ray crystallographic studies, and Table III compares the sensitivities of the Fe^{II}–CO, Fe^{II}–O_2, Fe^{III}–CN, and Fe^{II}–NO bond lengths to the trans ligand identity. Interestingly, the Fe^{II}–NO bond length is very similar for Fe^{II}(TPP)(*N*-MeIm)(NO) (1.74 Å)[3] and Fe^{II}(TPP)(4-methylpiperidine)(NO) (1.72 Å),[4] even though *N*-MeIm and 4-methylpiperidine are bases of different strengths. In contrast, the Fe^{II}–CO bond distance is different in Fe^{II}(deutero)(THF)-(CO) (1.706 Å)[52] and Fe^{II}(TPP)(pyridine)(CO) (1.77 Å).[31]

Similarly, the steric trans effect which is observed for CO, O_2, and CN complexes of iron porphyrins is absent for the NO case. For example, the $\nu(Fe^{II}–O_2)$ stretching frequency for solid Fe^{II}-(TpivPP)(*N*-MeIm)(O_2) appears 10 cm^{-1} higher than that of the sterically hindered Fe^{II}(TpivPP)(1,2-DiMeIm)(O_2) complex,[51] while the $\nu(Fe^{II}–NO)$ stretching frequency is observed at 524 cm^{-1} for both Fe^{II}(OEP)(*N*-MeIm)(NO) and Fe^{II}(OEP)(1,2-DiMeIm)(NO). Results from previous RRS studies of Mn^{II}-(heme-5)(*N*-MeIm)(NO) (629 cm^{-1}) and Mn^{II}(heme-5)(1,2-DiMeIm)(NO) (630 cm^{-1})[50] indicate that the strong Mn^{II}–NO bond of this system is also unaffected by alterations in the steric properties of the proximal base.

Table III. Comparison of the Trans Effect for CO, O_2, CN, and NO Complexes of Iron Porphyrins: Results from X-ray Crystallography

complex	Fe^{II}–CO bond length (Å)	ref
Fe^{II}(deutero)(THF)(CO)	1.706	53
Fe^{II}(TPP)(pyrdine)(CO)	1.77	31
complex	**Fe^{II}–O_2 bond length (Å)**	**ref**
Fe^{II}(TpivPP)(N-MeIm)(O_2)	1.75	54,55
Fe^{II}(TpivPP)(2-MeIm)(O_2)	1.90	56,57
complex	**Fe^{III}–CN bond length (Å)**	**ref**
Fe^{III}(TPP)(pyridine)(CN)	1.908	61
Fe^{III}(TPP)$(CN)_2$	1.975	62
complex	**Fe^{II}–NO bond length (Å)**	**ref**
Fe^{II}(TPP)(4-MePip)(NO)[a]	1.72	4
Fe^{II}(TPP)(N-MeIm)(NO)	1.74	3

[a] 4-MePip = 4-methylpiperidine.

Table IV. Solvent Dependence of the $\nu(Fe^{II}–NO)$ Stretching Frequency

solvent	dipole moment (D)	complex	$\nu(Fe^{II}–NO)$ (cm^{-1})
CCl_4	0	Fe^{II}(OEP)(pyridine)(NO)	523
C_6H_6	0	Fe^{II}(OEP)(pyridine)(NO)	522
$CHCl_3$	1.01	Fe^{II}(OEP)(pyridine)(NO)	524
CH_2Cl_2	1.60	Fe^{II}(OEP)(pyridine)(NO)	527
C_4H_8O	1.63	Fe^{II}(OEP)(THF)(NO)	523

These results are in conflict with those from earlier EPR studies of Fe^{II}(protoheme)(base)(NO),[19,57] in which both basicity and steric hindrance of the trans base were found to influence hyperfine structures. This was interpreted as an indication of trans effects on the Fe^{II}–NO bond. Discrepancies between RRS and EPR data have been noted previously in studies of nitrosyl complexes of metalloporphyrins.[19,58,59] It seems that, while EPR reflects only the distribution of the unpaired electron, the resonance Raman vibrational frequencies are a measure of the metal–ligand bond strength to which both s and p bonding contribute. Hence, hyperfine constants in EPR spectra are not necessarily a measure of metal–ligand bond strength.

Solvent Effect. It has been predicted[60] that, in simple "flat porphyrins" such as Fe^{II}(OEP)(base)(NO), the ligand will be exposed to the solvent and show a marked dependence on its physical properties. In this regard, we investigated the effect of the solvent on the $\nu(Fe^{II}–NO)$ stretching frequency, and these results are shown in Table IV. Apparently, nitric oxide does interact with the solvent. Dipole moments of the solvents are as follows in parentheses: CCl_4 (0 D), C_6H_6 (0 D), $CHCl_3$ (1.01 D), CH_2Cl_2 (1.60 D), and C_4H_8O (1.63 D). In general, the $\nu(Fe^{II}–NO)$ stretching frequency for solvents of higher polarity was higher, with THF providing a notable exception to this trend.

Conclusions

We report the first detection of the $\nu(Fe^{II}–NO)$ and $\nu(Fe^{III}–NO)$ stretching vibrations in protein-free porphyrins. This result

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is significant in two different aspects: (1) First, a comparison of the $\nu(Fe^{II}–NO)$ and $\nu(Fe^{III}–NO)$ stretching frequencies in porphyrins and hemoproteins is now feasible. While $\nu(Fe^{II}–NO)$ is observed $\sim 30\ cm^{-1}$ lower in porphyrins than in hemoproteins, the effects of the protein on $\nu(Fe^{III}–NO)$ are small. (2) Second, the detection of $\nu(Fe^{II}–NO)$ in model porphyrins enabled us to study trans effects on this bond. The $Fe^{II}–NO$ bond strength was determined to be independent of the electron-donating and stereochemical properties of the trans base. Clearly, the $Fe^{II}–NO$ bond is unique in this respect, and future work may be directed toward understanding this system more fully.

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